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EFFECT ON MYCORRHIZAL COLONIZATION ON ADDITION OF ZINC AND CADMIUM LEVELSIN *ELEUSINE CORACANA* L.

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ABSTRACT

A pot culture experiment was carried out to studythe effects of Arbuscular Mycorrhizal fungi on Finger millet (*Eleusine coracana* L.) mycorrhizal colonization, Zinc and Cadmium uptake under increasing Zn and Cd levels using a factorial design with 4X4 treatments with four levels of strains of mycorrhizal fungi viz.uninoculted control (M_0), inoculation with *Gigaspora* ACP-1(M_1), *Glomus* ACP-2(M_2) and *Scutellospora* ACP-2(M_3) and four levels of heavy metal each ofzinc sulphate at the rates of 0, 150, 300 and 450 mg Zn kg^{-1} soil and Cdat the rates of 0,25,50 and 100 mg Cd Kg^{-1} soil to find out the effects of inoculation with mycorrhizal strains under doses of Zn and Cdon finger millet (*Eleusine coracana* L.) at 30,60 and 105 days after sowing (DAS). In absence of Zn and Cd all the three fungal strains were equally effective in promoting plant growth, nutrients uptake and root colonization. When Zn and Cd were added to soil, the plant growth, uptake and mycorrhizal root colonization of nutrients decreased over control. *Glomus* ACP-2 (M_2 strain) was found to be the most tolerant strain to the increasing levels of Zn and Cdaddition to soil over the other two strains.

KEYWORDS: Arbuscularmycorrhizal, Cadmium, Fungi, Finger Millet, Soil Pollution, Zinc

INTRODUCTION

A mycorrhizal fungus is a symbiotic association in and on the roots of a host plant. ArbuscularMycorrhizal Fungi (AMF) are the most common group of mycorrhizal fungi, which are obligatory (Javaid, 2007) and they are found in more than 80% of land plant families (Smith & Read, 2008). The fungus is supplied with soluble carbon sources by the host plants, whereas the fungus provides the host plant with a better ability to take up water and nutrients from the soil (Javaid, 2009). Fungal hyphae not only speed up the nutrition state of the host plant but they also relocate heavy metals to the plant (Li *et al*., 2009) and protect the host plants from both biotic (Javaid, 2007) and abiotic stresses (Khaosaad *et al*., 2007). Although global deposition of some heavy metals is decreasing, local and global changes in soil conditions can contribute to mobilization of these potentially harmful metals. Arbuscularmycorrhizal (AM), fungi plays an essential role in providing access to minerals nutrients at all the stages of plant development. This is achieved largely through their ability to mobilize key nutrients such as phosphorus and nitrogen, consequently mycorrhizal fungi also influenced plant fitness and survival and the development of plant community structure. High concentration of heavy metals in the environment, cause a probable health threat in the long term. Contaminant metals accumulate in plant tissues and animal system. Plants take up heavy metals from their surroundings through a number of different plasma membrane localized transporters. Inside the plant system toxicity of heavy metals is manifested by an array of physiological and metabolic disturbances if metal detoxification processes are delayed or not efficient **(**Mishra and Dubey, 2006**)**. With the advent of environmental problems originating from pollutants having been transmitted up to the constant food chain, the concept of

ecological pollution has been the focal issue. Today, agriculture practices being full environmental concern into account are of great importance. This phenomenon has urged consistent cultivation system to be developed and implemented. The purpose of agri ecosystem is to ensure alternatives with the population of natural species is to be sustained and the likelihood hazardous effect is to be minimized. One of the alternatives proposed is that soil microorganism might be used in agriculture; thereby natural protection may be promoted. Arbuscularmycorrhizal (AM) fungi are among the most common of all soil fungi and they form symbiotic association with the roots of most vascular plants. However, little is known about their potential to adapt to toxic metals concentrative in soil. Heavy metals have been regarded to reduce or completely eliminate AM infection of plant roots in pot experiments, often at concentrations where phytoxic effects are not observed (Wang and Chao, 1991).During this environmental stress condition roots release greater quantities of organic compounds in the rhizosphere (Whipps, 1990) which relieve the external stress. The releasing of organic acids from the root is a general mechanism for solubilizing metals and act as a detoxification mechanism in acid soils (Jones and Darrah, 1994). AM fungi excrete organic acids such as citric, malic and oxalic acids into the rhizosphere (Landeweert *et al*., 2001). Root exudates are the first plant signals that AM fungi perceive and thought to play an important role in establishment of AM symbiosis. The root exudates of AM contain many organic compounds such as amino compounds, organic acids, fatty acids, sterols, flavones etc. which helped in the heavy metal sequestration. Among soil microorganism mycorrhizal fungi are the only ones providing a direct link between soil and roots therefore, is of great importance in heavy metal availability and toxicity to plants (Leyval *et al.,* 1997). The objective of this study was to investigate the possibilities of utilization of AM fungi in mitigating pollution in soil.

MATERIALS AND METHODS

A pot experiment was conducted to study the effects of inoculation of AM fungi (uninoculated as control) inoculated with *Gigaspora*ACP-1 (M_1), *Glomus*ACP-2 (M_2) and *Scutellospora* ACP-2 (M_3) obtained from soil microbiology laboratory, G. B. Pant University of Agriculture and Technology, Pantnagar, India. These were multiplied under sterile conditions with wheat as a host plant for 45 days. At the same time, the control mycorrhizal inoculums were also prepared under same conditions. The inocula were air dried and sieved (2mm) and each consisted of a mixture of rhizospheric soil from pure pot culture containing spores, hyphae and mycorrhizal and root fragments (the control without AM fungal propagules). The soil used in the study was collected from Crop Research Centre, Pantnagar B_6 plot. It had pH 7.32, 10.1 Kg ha^{-1} available P (Olsen *et. al.,* 1954), 1.17 organic carbon %, 4.40 µg g^{-1} DTPA extractable Zn (Lindsay & Marvell, 1969). The soil was sieved (4mm) and mixed with sand in 3:1 ratio. The different metal adding aqueous solution of zinc sulphate at the rates of 0, 150, 300 and 450 mg Zn Kg^{-1} soil and Cd @0,25,50,100 mg Cd Kg^{-1}soil. The metal adding aqueous solution of cadmium sulphate was soilCadmium at the rates of 0, 25, 50,100 mgCd Kg^{-1} soil. After carefully mixing the metal solution with the soil this was allowed to stabilize for 15 days before use.100 AM spores were placed in pot of 5 Kg capacity below 2 cm soil surface. A basal dose of 40 N Kg ha^{-1}, nitrogen in solution in soil in form of $(NH_4)_2$ SO_4 and potassium @ 20 K_2O Kg ha^{-1} as (KCl) were given in all treatments. Finger millet variety VL 146 was after surface sterilization of finger millet seeds with 0.5 % sodium hydrochloride for 15 minutes and three replications per 16 treatments were established. Plant heights at 30, 60 and 105 (maturity) days after sowing were recorded. The dry plant material was digested in diacid HNO_3:$HClO_4$ (9:4) ratio and extracts were used to estimate nutrient content in plant material. Initial soil samples and post-harvest soil samples were analyzed for available nitrogen (Subbiah and Asija, 1956, available phosphorus (Olsen *et.al;* 1954); available potassium, (Hanwayand Heidel, 1952) and DTPA extractable Zn

(Lindsay & Marvell, 1969).

RESULTS AND DISCUSSIONS

Mycorrhizal Colonization under Added Cadmium

Figure 1a showed effects of AM fungal root colonization ranged from 1.16 to 57.76 per cent at 60 DAS. A perusal of data indicates that inoculation with AM strains significantly increased mycorrhizalroot colonization at all levels of Cd concentrations in soil. Application of Cdalone up to 25 mg Kg^{-1} soil caused a non-significant reduction in root colonization by native mycorrhizal fungi. However, a significant reduction was recorded at application of 50 and 100 mg $CdKg^{-1}$ alone. The inoculation with *Gigaspora*ACP-1 (M_1), *Glomus*ACP-2 (M_2), *Scutellospora* ACP-2 (M_3) strains of mycorrhizal fungi individually increased about 48.63, 57.76 and 50.5 per cent root colonization respectively which were significantly higher over the absolute control. Application of doses of Cd successively decreased the infective capacity of all the introduced mycorrhizal fungi at 60 DAS. Among all strains, *Glomus*ACP-2 (M_2) strain was found to be most effective at all levels of Cd application. The sensitivity of AM endophytes to high amount of heavy metals expressed as reduction or delay of its colonizing ability has been reported by several workers (Gildon and Tinker, 1981).

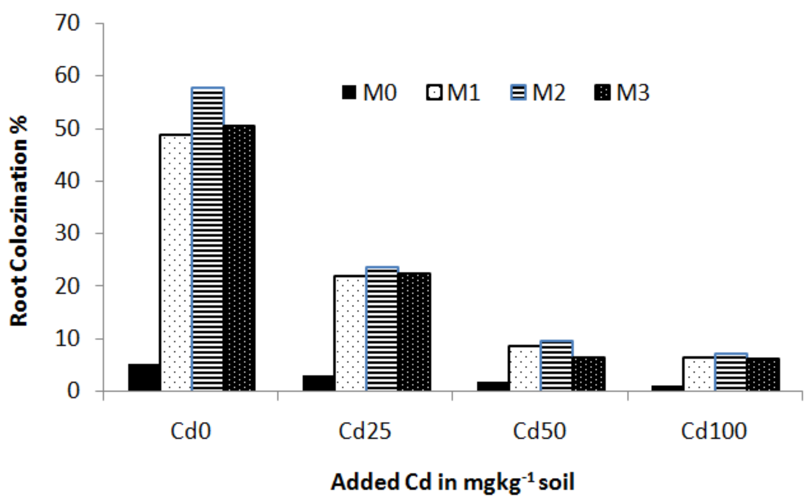

Figure 1a: Effect of Inoculation with Strains of Mycorrhizal Fungi, Cadmium Levels and Their Interactions on Mycorrhizal Root Colonization (%) At 60 DAS

Mycorrhizal Colonization under Added Zinc

Figure 1b showed effects of AM fungal root colonization ranged from 2.33 to 58.60per cent at 60 DAS. A perusal of data indicates that inoculation with AM strains significantly increased mycorrhizal root colonization at all levels of zinc concentrations in soil. Application ofZn alone up to 25 mg Kg^{-1} soil caused a non-significant reduction in root colonization by native mycorrhizal fungi. However, a significant reduction was recorded at application of 300 and 450 mg Zn Kg^{-1} alone. Among all strains, *Glomus*ACP-2 (M_2) strain was found to be most effective at all levels of Zn application. The sensitivity of AM endophytes to high amount of heavy metals expressed as reduction or delay of its colonizing ability has been reported by (Gildon and Tinker, 1981). Even though metals can exhibit a range of toxicities toward soil microorganisms AMF isolates, particularly the ecotypes living in metal-enriched soils, metallic ferrous sites and mine spoils heavily polluted with metals, can, depending on intrinsic and extrinsic factors, tolerate and accumulate HMs (Gildon and Tinker, 1981).

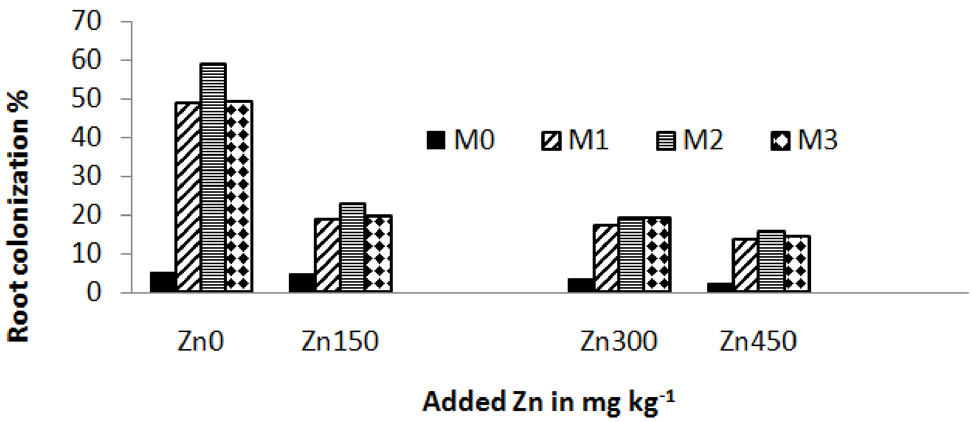

Figure 1b: Effect of Inoculation with Strains of Mycorrhizal Fungi, Cadmium Levels and Their Interactions on Mycorrhizal Root Colonization (%) At 60 DAS

Available Cadmium and Zinc inSoil

The available Cd ranged from 0.21 to 5.05 mg Kg^{-1} soil and available zinc ranged from 10.00 to 100.32 mg Kg^{-1}at the maturity of the crop (Table 1a &1b).With increasing levels of cadmiumand zinc there was a significant and successive increase in available cadmiumand zinc in soil over the control at 5% level of significance. Available cadmium in soil was significantly increased due to inoculation with different mycorrhizal strains over the uninoculated control (M_0Cd_0) strains, maximum being with M_2 strains followed by M_1 and M_3 at maturity. Whereas, available zinc in soil was significantly increased due to inoculation with different mycorrhizal strains over the uninoculated control (M_0Zn_0) strains, maximum being with M_2 strains followed by M_1 and M_3 at maturity. Interactions between strains of mycorrhizal fungi and doses of cadmium and zinc resulted significant increase in available Cadmium in soil over the absolute control. Increase in available soil cadmium and zinc due to inoculation with strains of mycorrhizal fungi individually may be attributed to the increase in the area of mycorrhizal fungi as compared to uninoculated control. These results are also corroborated with the results obtained by other researchers (Leyval *et. al*., 1997).Metal addition negatively affected nutrient uptake by mycorrhizal plants. The abundance of external mycelium produced by the AM fungi can be important for the heavy metal fixing ability of the fungi and consequently for their plant protecting action.

Table 1a: Effect of Inoculation with Strains of Mycorrhizal Fungi, Cd Levels and Their Interactions on Available Cd in Soil

Strains	Available Cd in Soil (mg Kg^{-1})				
	Cd_0	Cd_{25}	Cd_{50}	Cd_{100}	Mean
M_0	0.21	2.12	3.93	5.05	2.87
M_1	0.44	2.26	4.07	3.73	2.59
M_2	0.46	2.41	4.08	4.01	2.74
M_3	0.43	2.25	3.79	3.87	2.58
Mean	0.35	2.26	3.96	4.16	-
	M		Cd	M×Cd	
SEm±	0.03		0.03	0.07	
CD (P=0.05)	0.15		0.15	0.30	

Table 1b: Effect of Inoculation with Strains of Mycorrhizal Fungi, Zn Levels and Their Interactions on Available Znin Soil

Strains	Available Zn in Soil (mg Kg^{-1})				
	Zn_0	Zn_{150}	Zn_{300}	Zn_{450}	Mean
M_0	10.00	45.13	75.02	100.32	57.00
M_1	16.03	50.20	61.08	90.03	54.33
M_2	21.14	56.04	65.33	92.12	58.65
M_3	18.00	53.12	60.00	94.05	56.29
Mean	16.29	51.22	65.35	94.13	-
	M	Zn	M×Zn		
SEm±	0.84	0.84	1.68		
CD (P=0.05)	3.26	3.26	6.52		

CONCLUSIONS

It can be concluded that heavy metal contamination caused by either natural processes or by human activities is one of the most serious environmental problems. Among the soil microorganisms, Arbuscular mycorrhizal fungi provide a direct link between soil and roots, thereby enhancing the uptake of nutrient by the host plant. They are reported to be present on metal-contaminated sites and therefore to be of great importance in metal uptake by plant roots. Inoculation with AM fungi protects plant from the potential toxicity caused by cadmiumand zinc but the degree of protection varies according to the fungus plant combination. However, more knowledge of the mechanisms for how AM fungi contribute to plant growth and the absorption and translocation of heavy metal is necessary before application of inoculation can be considered in soil phyto- remediation programs. Further, the effect of AM fungi on the heavy metal uptake of the host plant depends on the physical and chemical properties of the contaminated soil and on the heaviness and duration of the contaminated load on the host plant and the fungal species.

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